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Swimming behavior of the aquatic larva of *Neoneuromus ignobilis* (Megaloptera: Corydalidae: Corydalinae)

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Abstract: In order to explore the pattern and significance of swimming, through photos and videos we observed and recorded the swimming behavior of the aquatic larvae of Megaloptera in detail for the first time using the endemic Chinese species *Neoneuromus ignobilis* Navás, 1932 as the test insect, which were collected from the Dadu River and reared in nature-simulated environments. Four swimming postures are recognized and described herein in detail, *i. e.*, vertical, parallel, back and side swimming, and these postures were used by the larvae disproportionately, with a frequency of 89.08%, 5.49%, 4.40% and 0.61%, respectively. The swimming larvae tend to pose their body into an S-shape, with various degree of sinuation. By changing the directions of the head and tail, they can easily rise up or sink and change swimming postures. The propulsion was generated by the wriggling of the body while the legs were mostly held close to the body. Larvae of different instars varied greatly in swimming ability, the 6th instar larvae being the best and most active swimmer compared to the 2nd and final instars. The larvae may also employ complex defense behaviors not often known from relatively ancient insect groups, like chemical defense as secretion from the end of abdomen.

Key words: Megaloptera; *Neoneuromus ignobilis*; aquatic insect; larva; swimming behavior; swimming posture

1 INTRODUCTION

Neoneuromus ignobilis Navás, 1932 (Corydalinae, Corydalidae, Megaloptera) is endemic to China (Liu, 2008). It is mostly aquatic in the larval stage, as is the case for all megalopteran insects (Yang, 1985; New and Theischinger, 1993). Larvae of Megaloptera are often sensitive to changes in water quality and thus are often considered to be useful indicators of water quality and valuable in environmental monitoring (Morse *et al.*, 1994; Elliott, 1996). Larvae of certain Megaloptera species have also been used as food, in traditional Chinese medicine, and as fish food in aquaculture of fish species with specialized feeding requirements (Liu, 2008).

In recent years, great progress have been made in studies on the taxonomy and systematics of the Megaloptera, especially from China and Southeast Asia, where the highest diversity of the order occurs (Cover and Resh, 2008; Liu, 2008). However, the biology of megalopteran insects, except for a few species, is generally poorly known (Smith, 1970; Smock, 1994; Elliott, 1996; Cover and Resh, 2008), and this situation is especially serious for the Chinese megalopterans (Wang *et al.*, 2001). The behavioral ecology, especially on swimming behavior, is characterized as a lack of details and existing only as brief, generic descriptions in the studies mentioned above.

In the present study, we report in detail the swimming behavior of the larvae of *N. ignobilis*. The larval development of Megaloptera typically consists of 10 – 12 instars and, for species whose larval development takes place in permanent water, the older larvae tend to move ashore, and subsequently pupate and emerge in the sand on the shore (New and Theischinger, 1993; Cover and Resh, 2008). With the aid of video filming and computer assisted analysis, we studied the swimming behavior of larvae of the 2nd, 6th and final instars of the species. Specifically we described in detail the swimming postures, swimming capability and speed, incentives for swimming, and responses to simulated stimuli and induced defensive swimming. Finally, we discussed the adaptive implications of the various aspects of the swimming behavior of the species in aquatic and terrestrial environments.

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2 MATERIALS AND METHODS

2.1 Test insects

All larvae of *N. ignobilis* used for this study were collected from the Dadu River in Leshan City, Sichuan Province, China and kept in a recovery tank similar to the aquarium described below filling to a depth of 40 cm with water maintained at 25 ± 2℃ and collected from the Dadu River. In addition to the microbes and invertebrates in the water already, a small amount of chironomid larvae were added in the water as additional food supply for the larvae of *N. ignobilis*.

2.2 Insect breeding equipment

A rectangular transparent glass aquarium measuring 70 cm × 50 cm × 30 cm was marked with smallest units of 0.1 cm at the bottom and on the sides for measuring the swimming speed of the larvae. As control, an aquarium was filled with clear water from the Dadu River to a depth of 15 cm, and a layer of 20 mm thick sand was placed on the bottom dotted with more than 10 small rocks. This way, we were able to create a habitat favorable to the larvae so that their swimming behavior was observed in simulated natural conditions.

2.3 Observation and determination of swimming behavior

The larvae used in this experiment were in one of the three age groups: 2nd instar, 6th instar, and the final instar that eventually entered pupation without further moulting. Our preliminary data indicated that *N. ignobilis* has 12 larval instars in Leshan area, but more strictly controlled rearing is needed to confirm this. At the beginning of the experiment, 20 larvae from one of the three age groups were gently placed in the experiment aquarium onto the water surface. The swimming behavior of the larvae was videotaped by a video camera (Fuji FinePix 2000HD) from the top and the side. Each shooting continued for 10 min and was repeated three times. The experiment was conducted on all three age groups and repeated five times each. In the end, a total of 300 larvae were used. The swimming behavior of the larvae was then analyzed using the video editing software Ulead (enhanced version) as described by Fu *et al.* (2005). In addition, the defensive swimming and related responses to external stimuli of the larvae were also studied by artificially stimulating the larvae using a glass rod.

3 RESULTS

3.1 Swimming postures of *N. ignobilis*

The swimming postures of the larvae of *N. ignobilis* are summarized into four categories, *i.e.*, vertical swimming, parallel swimming, back swimming, and side swimming. Each swimming posture is characterized in a certain degree of sinuation of the S-shape that the swimming larvae usually form by curling the body. Some of the swimming postures are more frequently observed than others.

3.1.1 Vertical swimming (Fig. 1: A). This was the most commonly used posture, being observed for about 89.08% of the time. It is mainly used for swimming up and down, and occasionally also for swimming forward and backward, but with much lower speed and mostly for maintaining in the same position. In vertical swimming, the longitudinal axis of body, from head to tail (the same below), was generally perpendicular to water surface, with head almost always positioned downward and the body forming a strongly sinuated S-shape. When the larva started to swim, its head was raised and bent downward abruptly, while its abdomen was strongly arched, at about the position of 4th – 5th segments, and then quickly straightened, forcing a downward current of water.

3.1.2 Parallel swimming (Fig. 1: B). This posture was observed for about 5.49% of the time, and was mainly used for swimming forward. In this posture, the longitudinal axis of body was parallel to water surface, with the body forming a slightly sinuated S-shape. Larvae swimming in this posture resemble a human swimming in breaststroke style – the head and abdomen strike the water gently to generate a forward push for the insect body at a speed of about 3.87 cm/s, the fastest among all the postures.

3.1.3 Back swimming (Fig. 1: C). This posture was observed for about 4.40% of the time, and was mainly used for retreating temporarily or changing postures, and occasionally for slow forward swimming. The longitudinal axis of body was generally parallel to or forming an angle of approximately 30° (or 150°) with the water surface. Larvae in this posture displayed a gently sinuated S-shape with the abdomen being relatively straightened. As a result, the route of swimming using this posture was horizontal or slightly oblique. Sometimes, the larvae would float in water in this posture for a long time, or coil up and stay afloat in water.

3.1.4 Side swimming (Fig. 1: D). This posture was the least commonly used, being observed for only 0.61% of the time. It was mainly used for

changing postures or swimming forward temporarily. Larvae would roll over at different angles. The longitudinal axis of body of the larvae was generally parallel to water surface, with the larvae swimming slowly in gently sinuated S-shape.

In addition, larvae of *N. ignobilis* would also swim in irregular or transitional swimming postures, which were observed for about only 0.42% of the time.

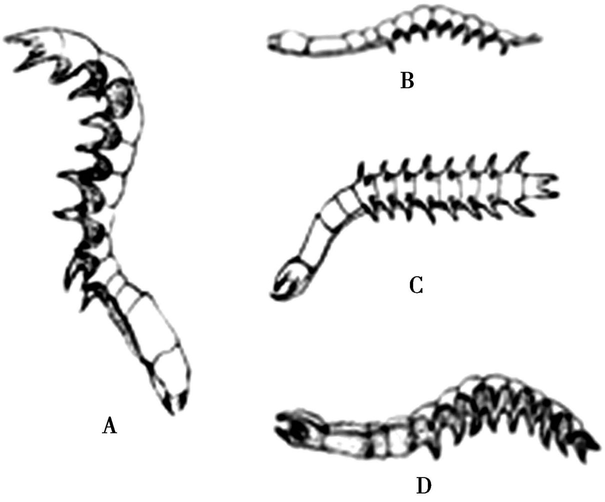

Fig. 1　Swimming postures of the larva of *Neoneuromus ignobilis* (right side view, legs not drawn)
A: Vertical swimming; B: Parallel swimming; C: Back swimming; D: Side swimming.

3.2 Upward and downward swimming of *N. ignobilis*

The larvae of *N. ignobilis* often remained a vertical or horizontal position before swimming. When a larva started to swim, it would raise its head first and then the abdomen without too much effort. Both of the head and the abdomen would strike the water to generate a push for swimming. The direction and frequency of the movements of the body determine the direction and speed of swimming.

The larvae of *N. ignobilis* usually set on the bottom of water in vertical, parallel and back swimming postures. The larvae would reduce the speed and magnitude of abdominal swinging, allowing them to sink slowly. The head would land first, then the legs, and finally the slender and long abdomen. Occasionally, a vertically swimming larva would halt all bodily movement, allowing itself to sink by gravity. On perceiving external attack, a larva would coil up immediately into a ball and allow itself to quickly settle to the bottom of water. Once on the bottom, it would stretch out the body and "return to life" again.

3.3 Change of swimming postures of *N. ignobilis*

The larvae of *N. ignobilis* changed their postures frequently while swimming, and the transition from one posture to the other was fast and smooth. Below are our observations of the details during transitions when the swimming larvae change postures.

3.3.1　Change between vertical and parallel swimming postures: This was the most commonly observed change, mostly from parallel swimming into vertical swimming posture. In the process, a slightly sinuated larva in the parallel swimming posture would wriggle its head and abdomen abruptly to adjust the angle between its body and the water surface while greatly increase the sinuation of the S-shaped body and strike the water more intensely. Change from vertical swimming to parallel swimming posture was also observed, but much less frequently. In this case, the movement of the head and the abdomen would first be subdued both in speed and in magnitude, and following the adjustment of the directions of the head and the abdomen. The larvae would then change into the parallel swimming posture.

3.3.2　Change between back swimming and parallel swimming postures: Larvae in back swimming posture was mainly propelled by the movement of the posterior part of the abdomen and the tail. In order to change the swimming posture from back swimming to parallel swimming, the movement of the posterior part of the abdomen and the tail gradually slowed down and the cephalothorax was suddenly turned around followed by the flipping over of the abdomen to finally bring the whole body to the parallel swimming posture. This change of swimming postures is easily reversed by a similar series of movements of the body parts.

3.3.3　Changing between parallel swimming and side swimming postures: For this change of swimming postures, the abdomen movement firstly slowed down, and then the cephalothorax rolled to the side, causing the abdomen to roll to the side as well and bringing the larva to side swimming posture. The change from parallel swimming to side swimming is easily reversed.

3.4 Body movement of *N. ignobilis*

The larva of *N. ignobilis* is able to curl its abdomen ventrally to form almost any angle with the anterior part of the body, or raise its abdomen dorsally to form a V-shaped angle with cephalothorax. In addition to flapping up and down, the abdomen is also capable of moving from side to side, thus changing the direction of swimming. On the other hand, all six legs of the larva are mostly

held close to the body (Fig. 2), with only slight variation in tightness at different swimming postures.

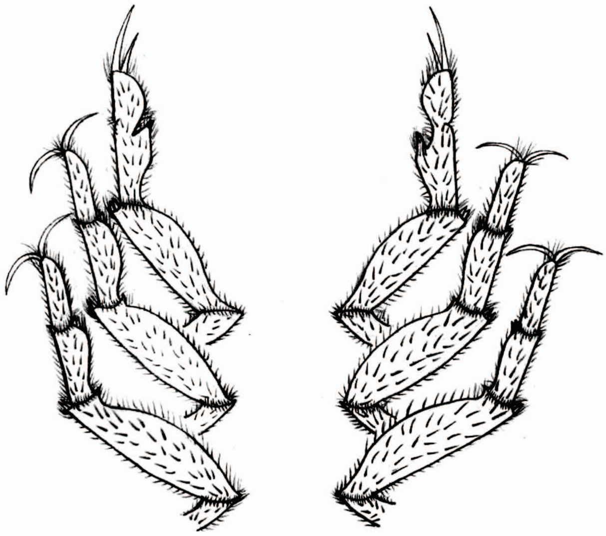

Fig. 2 Legs of swimming larvae of *Neoneuromus ignobilis* mostly folded close to the body

3.5 Swimming incentives of *N. ignobilis*

According to the observation results, the swimming activities of the larvae of *N. ignobilis* appeared to be mainly affected by five factors. 1) Changed environment – In a new environment that differed from the original one regardless of which compositions had changed, the larvae swam more frequently when there was enough water, and they would emit a lot of milky and water-insoluble substances from the end of their abdomen. The frequency of swimming would reduce significantly as they got used to the new environment. 2) Water space – The larvae tended to swim much more when there was plenty of water space, especially with increased depth. 3) External stimuli – The larvae swam more frequently when they encountered external stimuli that suggested attack or invasion. 4) Foraging swimming – The larvae occasionally swam for no obvious reason other than being in search for food. 5) Temperature – The larvae tend to hibernate during the winter and thus swam much less than during the summer, and they almost did not swim at all sometimes in the winter.

3.6 The swimming ability of *N. ignobilis* larvae at different instars

Our experiments showed that the 6th instar larvae were better swimmers than the larvae of the other instars, with an average continuous swimming endurance time of 78.5 s, an average forward speed of about 3.98 cm/s, and an average frequency of abdomen movement of 1.18 times/s, but the three numbers of 2nd and final instar larva being 60.8 s, 3.15 cm/s, 0.95 times/s and 62.4 s, 2.87 cm/s and 0.84 times/s, respectively. The maximum daily cumulative time of swimming of the 6th instar larvae was 23 min and 47.5 s as observed during the 14 hours of day time when the experiments were conducted (the larvae do not swim in the night). The swimming ability of the larvae of other two observed instars was considerably lower, especially the final instar larvae, which were not active and crawled, with a large body, at the water bottom.

3.7 The swimming behavior of *N. ignobilis* under the external stimulation

When disturbed by external stimuli, such as being touched by a stick, the larvae of *N. ignobilis* would curl up into a ball or immediately open the palates, ready to strike back an aggression. However, sometimes they would, after a quick immediate response, speed up and escape at a speed much faster than when undisturbed. In such a situation, the escape route was usually irregular with no fixed pattern, and the larvae would not maintain fixed postures, taking whatever postures that would allow them to escape effectively, such as swimming backward in parallel posture while attacking the disturbance objects using the palates.

4 DISCUSSION

Because the larvae of *N. ignobilis* live mostly in water, it is necessary for them to be powerful and efficient swimmers in order to escape from predators, search and hunt for food, disperse to new water space and avoid unsuitable water bodies in a timely manner. The rather powerful swimming capability, the flexibility of the various swimming postures and the easiness to change from one posture to another quickly and efficiently are obvious adaptations to the special environments where these larvae live.

However, the swimming ability of *N. ignobilis* larvae varied at different developmental stages, being relatively weak as younger larvae (2nd instar), became stronger later as mid-aged larvae (6th instar), and eventually decreased as they reached the final instar. This pattern of change in the swimming behavior of the *N. ignobilis* larvae is apparently a result of physiological limitations as well as an adaptive life history strategy. The larvae in the earlier instars are small and fragile with undeveloped muscles and thus have limited ability of swimming and are less likely to escape successfully from predation. The larvae gradually build up muscles and increase in strength, resulting in stronger swimming ability that improves their ability to hunt, defend, escape from predators, and disperse.

However, as they reach the last instar, they tend to come ashore, live in the mud and under the stone of the water banks to get ready for pupation. The water near the shore is usually not deep enough for larval swimming, and even in deeper water, due to significantly increased body weight, strength, and ability for self-defense and hunting, they no longer have the need for active swimming to escape from predators or to chase escaping preys when hunting. Consequently, a less mobile final instar larva is both necessary because of environment change and advantageous because of avoidance of unnecessary energy consumption.

The swimming power of larvae of *N. ignobilis* derives mainly from the movement of the body, particularly of the long abdomen. Adjustment of the direction, frequency and intensity of the water striking movement could result in forces varying in direction or strength and affect the posture, direction and speed of swimming. More pronounced sinuation of S-shape of the body would cause more forceful movement of the abdomen and thus generate more power. The high flexibility of the body, especially the abdomen, of the larva of *N. ignobilis* provides a physiological basis for the convenient change of swimming postures.

In addition, we also observed that the larvae would release considerable amount of milky and water-insoluble substances from the end of the abdomen when placed in a new environment. This may be related to chemical defense against predators, provoked by handling in our experiment, as observed in aquatic beetle (*Dineutes hornii*) (Eisner and Aneshansley, 2000). Alternatively, it may be a behavior similar to the secretion-grooming behavior of the water bug *Plea minutissima*, where the water bug used a chemical defense against microorganisms (Kovac and Maschwitz, 1989). Such a mechanism against microorganisms would be especially useful for an aquatic insect in a new environment.

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普通齿蛉幼虫的游泳行为

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摘要：为了探究广翅目昆虫幼虫在水中的游泳能力，以丰富其水生习性的行为学资料，选取中国特有种普通齿蛉 *Neoneuromus ignobilis* 幼虫为研究对象，通过室内试验对其游泳的姿势、刺激因素、不同龄期游泳能力及在外界刺激下的游泳行为进行了观察和测定。结果表明：普通齿蛉幼虫有垂直、平行、仰面和侧面等 4 种游泳姿势，出现的频率分别为 89.08%，5.49%，4.40% 和 0.61%。游泳时身体呈不同程度的“S”形，利用头部和尾部方向的改变实现虫体的上升、下沉和游泳姿势的改变。普通齿蛉幼虫利用身体的摆动游泳，游泳时 3 对足以固定的姿势靠紧身体。不同龄期的幼虫游泳能力差异很大，6 龄幼虫的游泳能力远强于 2 龄和末龄幼虫。在游泳时，普通齿蛉幼虫还具有比较复杂和独特的防御行为，如其腹部末端会喷射出化学物质。据此认为，普通齿蛉拥有较强的游泳能力，有助于其逃生和防御。

关键词：广翅目；普通齿蛉；水生昆虫；幼虫；游泳行为；游泳姿势

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